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A Review of the Disruptive Potential of Botulinum Neurotoxins as Chemical Warfare Agents

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14. ABSTRACT

The botulinum neurotoxins (BoNTs) act with high specificity and high potency to prevent neurotransmitter release at the neuromuscular junction, causing sustained paralysis, and at sufficiently high doses death by asphyxiation. The seven BoNT serotypes are the most potent substance known to man and are the only toxins designated Tier 1 select agents. Modeling of toxin distribution, mortality rates and first response limitations suggests that deployment of small amounts by terrorists could rapidly incapacitate the critical care infrastructure. Since BoNT is both a unique and valuable therapeutic drug as well as a highly potent weapon, the toxin is currently the only true "dual-use" select agent. The same characteristics that render the toxin a dangerous chemical weapon (persistence in vivo, exceedingly high potency, ease of distribution and ease of production) also make it highly effective for a broad range of clinical applications. The expansion in therapeutic BoNT uses is contributing to the increasing number of black market BoNT producers, which in turn raises the risk that technologies for synthesis, purification and distribution could be utilized to support political or economic terrorism. Below, we present a short review of the disruptive potential of the BONTs, emphasizing the nature of the security risk.

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"Paralyzed force, gesture without motion"
-The Hollow Men (1925), Thomas Stearns Eliot

1. Introduction. The flaccid paralysis caused by ingestion of botulinum neurotoxin (BoNT) was first described as "sausage poisoning" in 1820 and attributed to a bacterium in 1897 [1]. We now know that the toxin is a protein produced by *Clostridium botulinum* and currently comes in seven different serotypes designated /A to /G (e.g., BoNT/A represents the toxin produced by *C. botulinum* serotype A). Although the serotypes exhibit different host ranges and cellular targets, intoxication of susceptible hosts invariably results in neuromuscular paralysis.

The same characteristics that make the BoNTs the most lethal substances known (persistence *in vivo*, exceedingly high potency, ease of use and ease of production) also renders the toxin an effective pharmaceutical for a broad range of therapeutic and cosmetic uses [2-3]. BOTOX® (the brand name for the most common BoNT preparation) and its competitors are projected to net $1.5 to $2 billion in 2011 sales of cosmetic and therapeutic products [4]. The rapidly increasing list of off-label therapeutic uses for the toxin is contributing to the expanding number of black market BoNT producers, which in turn raises the risk that technologies for synthesis, purification and distribution could be utilized to support political or economic terrorism [5]. Although the total amount of toxin in a clinical preparation for cosmetic use is less than 1% of the dose necessary to kill a single person, production processes are effectively the same for bulk toxin and clinical grade toxin. In 2010, the Washington Post reported that an unlicensed purveyor of concentrated toxin was identified in Russia [6]. Although the man escaped and the (alleged) Chechnyan producer of that particular lot of toxin was apparently not located, the Post reported that US officials believed that "dozens of labs" located in Europe and Asia are supplying an expanding black market for BoNT (these estimates are consistent with a 2010 report commissioned by the DOD Defense Threat Reduction Agency [7]). As the article notes, "[BoNT] is the only profit-making venture for terrorists that can also potentially yield a weapon of mass destruction." Since BoNT is both a unique and valuable therapeutic drug as well as a highly potent weapon, the toxin is currently the only true "dual-use" select agent. The purpose of this report is to provide context as to the dangers presented by malicious use of BoNT.

2. BoNT Structure and Cellular Mechanism of Action. The BoNTs are relatively large heterodimeric proteins, comprised of a 50 kiloDalton (kDa) light chain (LC) and a 100 kDa heavy chain (HC). The LC is responsible for intracellular enzymatic activity, and the HC provides neuron-specific targeting and acts as a vehicle to transport the LC into the presynaptic compartment. Since BoNTs cannot penetrate the skin, the toxin must be internalized by ingestion, inhalation or injection to be dangerous. There are several "natural" ways to contract botulinum poisoning:

- Ingestion of preformed toxin, for example, by ingestion of foods in which spores have germinated under anaerobic conditions (typically home-canned; fermented, uncooked; or improperly cooked dishes);

- Ingestion of spores, which in turn colonize the small intestine (occurs in infants or clinical patients taking antibiotics that depopulate the gut flora); and
- Contamination of a wound by the bacterium, which then secretes toxin into the bloodstream.

After exposure by any of the above routes, BoNT gains access to the bloodstream and binds to neuron-specific receptors present on presynaptic terminals of neuromuscular junctions (NMJ). NMJs are cellular structures at which a nerve terminal synapses with a muscle fiber. Activation of the nerve terminal results in the release of chemical messengers that instruct the muscle fiber to contract. By binding to presynaptic receptors, BoNTs employ a Trojan horse-like strategy to gain access to the interior of the nerve terminal, whereupon the toxin targets one of three proteins for destruction. These proteins--SNAP-25, VAMP-2 and syntaxin--are essential to the release of neurotransmitter from the neuron [8-9]. Functionally, this means that neurons can no longer elicit muscle contraction, resulting in muscular paralysis. Once the nerves that control the diaphragm are intoxicated, emergent respiratory failure ensues and victims asphyxiate [9].

3. Medical Approaches to Protect from Intoxication. Counteragents to BoNT can either be delivered prophylactically (prior to the onset of symptoms) or therapeutically (after the onset of symptoms). Prophylactic resistance to several hundred LD_{50}s of toxin can be induced by a series of immunizations using inactivated toxin as an immunogen. Immunized persons can no longer be treated with BoNT for therapeutic or cosmetic purposes, so this approach is not widespread. Alternatively, short-term prophylactic protection can be provided by delivery of immunoglobulins harvested from previously immunized farm animals, which can scavenge BoNT while it is still in the vascular system. In animal studies, delivery of this "antitoxin" within 12 hours of challenge with over 1,000 lethal doses of BoNT has protected against paralysis. However, since the antitoxin is rapidly cleared from the body and is not effective against BoNT that has already bound to or been internalized into neurons, it only provides prophylactic protection. Due to the limited availability of the antitoxin, adverse events resulting from cross-species reactions and short-lived protection of antitoxin preparations, widespread antitoxin prophylaxis is impractical.

Once the toxin is sequestered within the presynaptic terminus, no therapeutic approaches are currently available to accelerate the recovery from paralysis. Identifying such therapeutic candidates is the purpose of our research program [10]. It is estimated that as few as 10-100 toxin molecules per synapse are sufficient to cause paralysis, which means that a therapeutic must be exquisitely potent and highly efficient to result in clinically effective inhibition of LC activity.

Clinical Presentation and Treatment of BoNT Intoxication. The first clinical evidence of botulinum intoxication generally appears within 1-2 days after exposure as an acute, symmetric, descending, flaccid paralysis with a normal sensorium. These symptoms are the result of toxin that has already been internalized into neurons and therefore cannot be reversed. Once evidence

of intoxication is present, clinical options are limited to supportive care and to inactivating residual toxin that may remain within the blood [11]. Victims receiving a lethal dose (described below) require ICU support, including a ventilator for respiratory support and a feeding tube for parenteral nutrition. Despite being paralyzed, patients do not suffer cognitive deficits and remain conscious, though unable to effectively communicate. Substantial resources are required to provide the full-time support necessary to care for these patients, and as few as a dozen paralyzed victims are likely to saturate the capacity of a mid-size city hospital ICU. Depending on the serotype, paralysis can persist for months, requiring sustained intensive medical care [12-13]. Furthermore, once the toxin is cleared from poisoned nerve termini, the synapse must be regenerated and coordinated neuromuscular control re-established. In a recent instance of BoNT exposure resulting from physician error, the patient required nearly a year to become ambulatory [14]. Even 5 years after the exposure, the victim still exhibits what appear to be residual morbidities, including muscle weakness, emotional distress and frequent headaches (personal communication).

4. Crisis Management and Deployment of BoNT as a Terror Weapon. Misuse of BoNT, either deliberate or accidental, is likely to result in a large number of patients simultaneously presenting with the early symptoms of intoxication. Since the first symptoms of intoxication manifest within 12-48 hours as a progressive paralysis which can easily be confused with a diversity of other illnesses, there is a high likelihood that early victims will suffer significant paralysis prior to clinical diagnosis. Since the antitoxin is most effective within 12 hours of exposure, timing is crucial in terms of reducing the extent of paralysis in the exposed population. Furthermore, due to the high potential of adverse events following immunization with a cross-species immunoglobin preparation, clinicians may be initially hesitant to apply antitoxin without strong epidemiological or clinical evidence of exposure. Limiting the number and severity of clinically intoxicated victims will, therefore, depend on how quickly medical personnel can work through the differential diagnoses, request and obtain antitoxin, identify the potentially exposed population and deliver the antitoxin, while simultaneously managing victims.

Consequently, effective casualty management procedures depend on the early identification and treatment of potential casualties while simultaneously providing emergency care to extant victims. The ability of caretakers to ensure that the exposed population is alerted and provided with a post-exposure prophylaxis are limited by at least two key delays: the period of time before a differential diagnosis is correctly made and the delay between diagnosis and the notification and monitoring of the exposed population. These problems become more complicated once the general population becomes aware of the emergency, and healthcare facilities are flooded by healthy persons seeking prophylaxis, despite being clinically asymptomatic.

Due to the high medical resource demands of paralyzed victims, emergency care providers and clinical ICUs will find it difficult to provide beds, ventilators, personnel and ancillary critical care needs without sacrificing other clinical services for the duration of the victim's residency. In such a scenario, those victims that become symptomatic the earliest are likely to suffer the

most severe paralysis. The resource bottleneck of any disaster response will be the availability of emergency personnel, ventilators and critical care beds, which can easily become overwhelmed. Thus, even a small-scale mass casualty event could rapidly disrupt the local health-care infrastructure. For anybody trained in epidemiology and crisis management, this presents a "devil's brew" of potential failure points and emphasizes the critical need for a therapeutic that offers symptomatic relief from BoNT-mediated paralysis.

A. How Severe a Threat Does BoNT Really Represent? It is a truism among the biodefense community that BoNT is the most toxic substance known. The dose of BoNT/A that will kill 50% of an exposed population is estimated from animal studies to be as low as 0.4 ng/kg body weight by intravenous administration, 3 ng/kg by inhalation and 50 ng/kg by ingestion. This means that for a 70-kg (155 lb) human, inhaling as little as 0.0002 mg (or 0.000000007 oz) of toxin is likely to result in mortality. BoNT/B and /E have similar potencies. The BoNTs are 10^3 to 10^6 times more lethal than other chemical warfare agents (such as ricin, sulfur mustard or nerve agents), resulting in their classification by the CDC as one of six Category A select agents, and the only Category A agent that is a toxin. The Category A agents have the highest priority for research and defense based on ease of dissemination, high mortality rates, the potential for major public health impact, the ability to cause public panic and social disruption, and the requirement for special action for public health preparedness [15].

B. Mathematical Models of a BoNT Deployment. Although malicious delivery of preformed toxin is possible by injection or aerosol inhalation, dispersal in food or liquid matrix may be a more plausible route for a mass casualty event. Hypothetical models describing the distribution of BoNTs in a liquid dispersal medium have been developed from real-life incidents of biotoxin exposure [16-17]. A more detailed mathematical exercise that highlights how toxin could be used in a terrorism event was presented by Stanford University Professor Lawrence Wein in a PNAS paper and a 2005 New York Times guest editorial [18-19]. Dr. Wein described procedures by which addition of gram-quantities of toxin to unpasteurized milk prior to delivery to a raw-milk silo could result in the exposure of 10-100 times more civilians than died in the terrorist attacks on September 11, 2001.

Some back-of-the-envelope calculations substantiate how the carefully planned delivery of a small amount of neurotoxin could rapidly overwhelm a municipality's emergency management system. For example, drinking 8 ounces of liquid tainted with 3.5 ug of BoNT/A would constitute a median human lethal dose. Addition of as little as 14 ug of toxin to a quart of liquid would be sufficient to achieve this concentration. Thus, delivery of one gram of toxin (equivalent to the weight of a paperclip, and relatively easy to generate from a toxic strain of *C. botulinum* with minimal training [6]) to a bulk liquid prior to distribution would be sufficient to contaminate approximately 69,000 quart containers. If we assume that the toxin is stable in the dispersal medium, then 50% of those that drink at least 8 oz would die within 2-3 days without medical support, and nearly all would require acute and prolonged medical care. Even if this

were only a regionally distributed item, one can imagine the widespread alarm and chaos once it became apparent that a commercial product had been tainted with a slow-acting, lethal toxin.

As noted in a rebuttal to Dr. Wein's paper, these scenarios include a number of simplifying assumptions, including the belief that unconventional actors would have the expertise to generate gram quantities of toxin [20]. However, given evidence of an increase in illicit producers of black market BoNT, the acquisition of knowledge and skills necessary for bulk production clearly does not present an insurmountable barrier, particularly to a state-supported organization [5]. In fact, a study by two biodefense researchers asserts that a trained laboratory technician can produce a gram of purified toxin for as little as $2,000.[1] Instructions on growing toxic strains and purifying toxin using household items are available from multiple places online.[2] Unlike infectious agents or chemical agents that have percutaneous activity, *C. botulinum* and purified toxin can be safely handled with relatively few precautions. A more sophisticated approach would obviate the need to culture a toxic strain by synthesizing a BoNT gene sequence from publicly available databases and expressing the recombinant gene in *E. coli* using commercially available bacterial expression vectors (a routine laboratory procedure). This would also enable the facile modification of the recombinant gene, for example to modify potency or escape biosurveillance. Although production of toxin with sufficient potency to enable a mass exposure is theoretically feasible with minimal training, we would argue that a far more significant threat is presented by malefactors with post-graduate training in the life sciences who have access to an equipped and functioning research facility.

If nothing else, Dr. Wein's paper described for the first time a critical security gap (which has since been mitigated) and invoked an extensive, ongoing debate in the biodefense community over what type of information should be presented in open scientific literature [21]. It should also be noted that some food products may be more amenable to contamination with active BoNT than milk-based dairy products, although for obvious reasons these will not be discussed here.[3]

6. Conclusions. The botulinum neurotoxins act with high specificity and high potency to prevent neurotransmitter release at the neuromuscular junction, causing sustained paralysis, and at sufficiently high doses, death by asphyxiation. The same characteristics that make the BoNTs such effective therapeutic tools also put them at high risk of misuse and have resulted in their classification as CDC Category A select agents. Mathematical modeling of toxin distribution and mortality rates suggests that deployment of as little as one gram by terrorists could result in 10^4-10^5 casualties. It is worth acknowledging that the modernization of epidemiological surveillance networks and emergency management plans since the American anthrax attacks of 2001 is likely to mitigate morbidity and mortality. Moreover, distributing sufficient toxin via

[1] information held by author.
[2] information held by author.
[3] information held by author.

contamination of commercial products, although feasible, would be a difficult undertaking and likely to be prone to failure.

A criticism of the argument that BoNT presents a significant bioterror threat revolves around the opinion that growing potent strains of *C. botulinum* and purifying sufficient toxin for a large-scale attack are more difficult than expected. These counterarguments are weakened by the transformative potential of modern molecular biology, the widespread availability of protocols, and the fact that a successful dissemination to even a small area would be highly disruptive to the economy and the medical infrastructure. In fact, contaminating a handful of products at several grocery stores by direct injection of toxin may have a disruptive effect while requiring small amounts of BoNT (less than the exempt limit) and would be relatively easy to execute without suspicion.

There is no public evidence of a successful bioterror attack against US citizens by a foreign agency following 9/11. This begs the question: if, as asserted, it is relatively easy to generate a Category A select agent such as BoNT at sufficient levels to enable its use as a terror weapon, why have such attacks not happened? In the absence of additional information we can only hypothesize. Perhaps it is merely a matter of time until such an attack occurs; perhaps we have an active, covert program that has successfully prevented such attacks from happening; perhaps those that provide direction to malefactors believe that a biological or chemical attack will mandate too severe of a response; or perhaps other, less technically intensive approaches are more suited to the current technological level of terrorist activities. Regardless, although exercises such as those presented by Dr. Wein incite controversy, they also illustrate that a successful exploitation of gaps in food security measures could have a crippling effect on our healthcare system, and would be likely to result in a transformative event that could exceed 9/11 in scope.

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